

Endomorphins: Novel Endogenous μ -Opiate Receptor Agonists in Regions of High μ -Opiate Receptor Density

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ABSTRACT: Endomorphin-1 (Tyr-Pro-Trp-Phe-NH₂, EM-1) and endomorphin-2 (Tyr-Pro-Phe-Phe-NH₂, EM-2) are peptides recently isolated from brain that show the highest affinity and selectivity for the μ (morphine) opiate receptor of all the known endogenous opioids. The endomorphins have potent analgesic and gastrointestinal effects. At the cellular level, they activate G-proteins (³⁵S-GTP γ -S binding) and inhibit calcium currents. Support for their role as endogenous ligands for the μ -opiate receptor includes their localization by radioimmunoassay and immunocytochemistry in central nervous system regions of high μ receptor density. Intense EM-2 immunoreactivity is present in the terminal regions of primary afferent neurons in the dorsal horn of the spinal cord and in the medulla near high densities of μ receptors. Chemical (capsaicin) and surgical (rhizotomy) disruption of nociceptive primary afferent neurons depletes the immunoreactivity, implicating the primary afferents as the source of EM-2. Thus, EM-2 is well-positioned to serve as an endogenous modulator of pain in its earliest stages of perception. In contrast to EM-2, which is more prevalent in the spinal cord and lower brainstem, EM-1 is more widely and densely distributed throughout the brain than EM-2. The distribution is consistent with a role for the peptides in the modulation of diverse functions, including autonomic, neuroendocrine, and reward functions as well as modulation of responses to pain and stress.

DISCOVERY OF ENDOGENOUS OPIOIDS

The first endogenous agonists for opiate receptors (enkephalins¹) were discovered in 1975. Examination of differences between morphine and enkephalins in their activities in bioassays led to the discovery of the δ -opiate receptor.² The enkephalins

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bound with greater affinity to the δ receptor than to the other two currently known opiate receptors, the μ and κ receptors. The enkephalins are therefore considered to be the endogenous agonists for the δ receptor. β -Endorphin, the second endogenous opiate-like agonist discovered,³ was found to bind with about equal affinity to the μ and δ receptors.⁴ Dynorphins, the last of the three currently well-known and characterized families of peptides, were discovered in 1979⁵ and preferentially bind to κ receptors.⁶

A common motif in all of these peptides is the signature N-terminus tetrapeptide Tyr-Gly-Gly-Phe. Based to some extent on this sequence homology, each of the three peptide families can “spill over” to at least one of the receptors other than its “preferred” site. Thus, enkephalins bind to μ receptors at a 10–20-fold lower affinity than to their preferred δ receptor. Dynorphin binds with a sixfold lower affinity to the μ site than to the κ site, and β -endorphin is indiscriminate in its binding to μ or δ receptors.⁴

A guiding concept leading to the discovery of endogenous opioid peptides was that it seemed unlikely that a receptor would be present in the nervous system only to respond to a ligand (morphine) derived from a plant (poppy), to which an organism may or may not ever be exposed. A more likely possibility was that the nervous system would produce a natural agonist for that receptor. The discovery of opiate receptors in the brain⁷ therefore stimulated the race to find the endogenous ligand, and within a short time, the peptides described above were discovered.

Based on a similar concept, a mystery remained after the discovery of the three families of opioid peptides. The μ -opiate receptor is essential to the analgesic and euphoric actions of morphine, as shown by the elimination of these effects in μ -opiate receptor knockout mice.^{8,9} It seemed unlikely that the only natural ligands for the μ receptor were those that either preferentially bind to other types of opiate receptors (enkephalin to δ and dynorphin to κ receptors) or indiscriminately bind to μ or δ receptors (β -endorphin). The plant-derived alkaloid, morphine, by contrast, binds to the μ receptor with nearly two orders of magnitude greater affinity compared with its next-preferred site, the δ receptor.^{4,10}

PEPTIDES SELECTIVE FOR μ RECEPTORS

Naturally occurring peptides have been found that preferentially bind to the μ opiate receptor. In contrast to the peptides discussed above, most of these peptides have an N-terminus Tyr-Pro sequence and include β -casomorphin (Tyr-Pro-Phe-Pro-Gly-Pro-Ile) from tryptic digests of β -casein,¹¹ hemorphin (Tyr-Pro-Trp-Thr)¹² from digests of hemoglobin, Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH₂)¹³ and Tyr-W-MIF-1 (Tyr-Pro-Trp-Gly-NH₂),¹⁴ both isolated from brain. Although an aliphatic amino acid in the third position can confer selective binding to the μ receptor, as with Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH₂),¹⁵ an aromatic (Trp or Phe) amino acid in this position provides higher affinity binding.¹⁰ For all of these peptides, however, the affinity for the μ receptor was well below that of the familiar endogenous opioids. The highest affinity (20–50 nM) exogenous peptide was the casomorphin-derived peptide morphiceptin, whereas the highest affinity endogenous peptide was Tyr-W-MIF-1 (70 nM).¹⁰

DISCOVERY OF ENDOMORPHINS

Based on knowledge of this "Tyr-Pro-aromatic" motif for μ selectivity, we used the brain peptide Tyr-W-MIF-1 (Tyr-Pro-Trp-Gly-NH₂) as a parent compound to search for a higher-affinity natural ligand.¹⁶ We systematically substituted each of the 20 natural amino acids for the Gly in position 4 and tested these analogues for binding to the μ receptor. Although most of the analogues showed affinities in the range of 0.2–6 times higher than that of Tyr-W-MIF-1, the analogue with Phe in position 4 (Phe⁴) showed an affinity of more than an order of magnitude higher than the other analogues. The selectivity for binding to the μ receptor relative to the δ or κ receptor also was dramatically increased, with an affinity ratio of more than three orders of magnitude. Charged amino acids inhibited the binding, but the hydrophobicity of the amino acid in position 4 correlated with binding. The binding of the Phe⁴ analogue, however, showed a significant deviation from this correlation, indicating that the dramatic increase in binding could not have been predicted from theoretical models based on charge or hydrophobicity.

The high-affinity binding of this newly characterized peptide (Tyr-Pro-Trp-Phe-NH₂) did not necessarily mean it would have agonist properties. Furthermore, just because it was composed of natural amino acids did not mean that the nervous system produced it. The former issue was resolved by the demonstration that the peptide was extremely potent in the guinea-pig ileum assay, the classical test of opiate agonist activity.¹⁶ It was more active than the potent enkephalin analogue DAMGO, and this effect was reversed by the μ antagonist CTOP, reflecting its selectivity for the μ receptor. The analogue also had potent and specific agonist action *in vivo* as shown in the tail-flick test. The antinociceptive potency after intracerebroventricular (i.c.v.) injection rivaled that of morphine and was reversed by the specific μ antagonist β -funaltrexamine. The peptide was even more potent after intrathecal (i.t.) injection than after i.c.v. injection.

The question of whether the nervous system could produce such a peptide was addressed by the generation of a specific antibody against it and use of that antibody to screen fractions of bovine brain extract purified by HPLC. After several steps of separation, a purified immunoreactive fraction was subjected to Edman degradation sequencing to reveal the presence of two peptide sequences: Tyr-Pro-Trp-Phe-NH₂ and Tyr-Pro-Phe-Phe-NH₂. With only slightly lower affinity than the first sequence, the second also was found to bind with subnanomolar affinity and >1000-fold selectivity for the μ receptor and to have potent agonist activity in the ileum assay and tail-flick test. Thus, the brain extract contained two previously unknown opioid peptides. Subsequently, similar isolation procedures with extracts of human brain¹⁷ revealed that both peptides also were present in human brain and in greater quantities than in the bovine brain where they were originally discovered.¹⁶

One of the original names proposed for endogenous morphine-like compounds was the contraction "endorphin." This term was not chosen, however, as the name for the first opiate-like peptide discovered, enkephalin. Subsequently, β -lipoprotein was found to contain a sequence with potent opiate-like activity that was named β -endorphin.³ Although it was proposed in 1983 by a committee of the prestigious International Narcotics Research Conference (INRC) to restrict the term endorphin to refer to the specific peptide β -endorphin, the term endorphins has commonly been

used within the scientific community as well as in the general public as a generic term for all of the endogenous opioids. This original contraction, proposed by Eric Simon, did not include an “m”, in part to clearly distinguish the endogenous compounds from morphine. This may in retrospect have been appropriate: None of the three previously known peptide families bound preferentially to the μ receptor, although each of them could activate the site as described above. Because the two newly discovered peptides (Tyr-Pro-Trp-Phe-NH₂ and Tyr-Pro-Phe-Phe-NH₂) were endogenous peptides that had the highest affinity and selectivity for the μ receptor, they were named endomorphin-1 and -2 (EM1 and EM2).

CELLULAR ACTIONS OF ENDOMORPHINS

In addition to the *in vitro* effects in the ileum and the i.c.v. and i.t. effects on analgesia described in the original paper, post-receptor cellular actions of the peptides also were consistent with their μ agonist profile. A major effect of opioids is to inhibit cellular excitability. One mechanism by which this is achieved is by inhibition of calcium currents. When applied to neuroblastoma cells transfected with the human μ -opiate receptor, the endomorphins showed a dose-dependent, naloxone-reversible inhibition of voltage-dependent calcium channels.¹⁸

The μ receptor is a G-protein coupled receptor, so the signal transduction cascade of μ receptor agonists begins with activation of G-proteins. When applied to membranes of μ -receptor-containing SH-SY5Y human neuroblastoma cells, the endomorphins stimulate the binding of ³²S-GTP- γ -S to the membranes.¹⁹ This relatively recent, but now well-established, test for activation of G-proteins also revealed that the endomorphins have lower efficacy than DAMGO, the standard high-efficacy μ agonist that is the reference compound in this assay. This difference in efficacy may have implications for the susceptibility of the agonists to desensitization. It may be, for example, that the lower efficacy of the endomorphins could make them more resistant to loss of responsiveness with repeated or prolonged exposure. The effect of endomorphins compared with DAMGO in the GTP- γ -S binding assay, however, is in contrast to that observed in the guinea-pig ileum, where the endomorphins were significantly more potent than DAMGO.¹⁶ The basis for these differences is unclear at this time.

MAPPING THE DISTRIBUTION OF THE ENDOMORPHINS

Endomorphin-2 in the Spinal Cord and Brainstem

Although exogenous application of peptides in the various tests described thus far is very useful for the determination of their binding and agonist characteristics, an understanding of the natural endogenous role of the peptides depends upon a detailed characterization of their distribution in the nervous system. A crucial criterion for characterizing the peptides as endogenous agonists for the μ receptor is that the neuronal processes releasing them should be anatomically localized near neurons expressing the μ receptor. The initial report of the discovery of the endomorphins demonstrated by RIA that the peptides were present in areas important for μ ac-

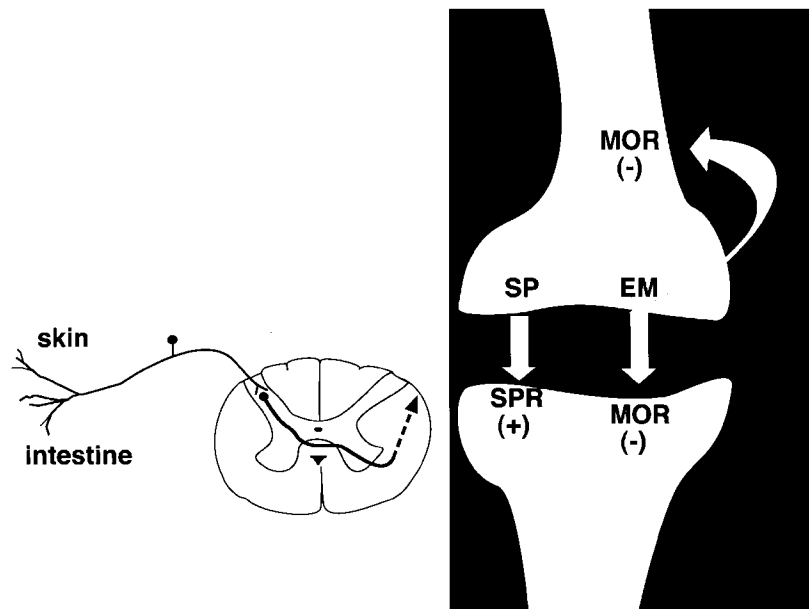


FIGURE 1. Hypothetical role of endomorphin (EM) in the modulation of nociceptive input at the synapse of primary afferents in the dorsal horn. Activation of presynaptic μ opiate receptors (MOR) could inhibit (–) the release of neurotransmitters such as substance P (SP), reducing the activation of the SP receptor (SPR) that normally has excitatory (+) effects on postsynaptic cells. EM could also act at postsynaptic MOR to decrease excitability in these cells.

tions.¹⁶ The first detailed immunocytochemical demonstration that an endomorphin was present in close proximity to μ receptors showed relatively intense staining of EM2-like immunoreactivity (EM2-LI) in the superficial layers of the dorsal horn in the spinal cord and medulla and in fibers within the dorsal root ganglia.²⁰

The dorsal horn has long been known to contain one of the highest densities of μ -opiate receptors in the nervous system and is an important site for the analgesic effects of morphine. Severing the primary afferents (rhizotomy) dramatically reduces, but does not eliminate, μ receptors in the dorsal horn.²¹ This is consistent with the idea that μ receptors are present both on the dendrites of the cell bodies within the dorsal horn of the spinal cord, as well as on the terminals of the primary afferents that originate in the dorsal root ganglia. A long-standing question concerning these presynaptic receptors, however, is what natural agonist activates them. The dynorphin- and enkephalin-expressing neurons present in the dorsal horn make few synaptic connections with primary afferent axons containing μ receptors, and they most likely modulate nociceptive input by postsynaptic rather than presynaptic mechanisms (for review, see ref. 22). It also is unlikely that these opioids serve an autoregulatory function: enkephalin is not present in a significant population of primary afferents and is consistently unaffected by rhizotomy. The effects of rhizotomy on dynorphin are inconsistent, and dynorphin immunoreactivity that is colocalized with substance P (SP) is unaffected by rhizotomy (for reviews see refs. 22 and 23).

By contrast, a substantial body of evidence supports the idea that EM2 is present in primary afferents where it can serve both an autoregulatory function (by activating the μ receptors on primary afferents), as well as function to regulate the excitability of postsynaptic cells (by activating the μ receptors on cells within the dorsal horn). EM2-LI is colocalized with SP-LI in a subset of SP fibers,^{20,22} and with CGRP-LI.²⁴ These are the two major excitatory peptide transmitters responsible for transmission of nociceptive signals from the primary afferents to the projection neurons and interneurons in the dorsal horn. EM2 is also colocalized with the μ receptor in some fibers in the dorsal horn.²² Unilateral dorsal rhizotomy dramatically reduces EM2 immunoreactivity in the dorsal horn only on the rhizotomized side.^{22,24} Capsaicin selectively activates and, in high doses, can ablate nociceptive primary afferent C- and A- δ fibers.²⁵ This selective neurotoxin virtually abolished EM2 staining in the dorsal horn of the spinal cord and medulla.²² Thus, disruption of primary sensory afferents by mechanical (rhizotomy) or chemical (capsaicin) methods essentially abolished EM2-like immunoreactivity. This body of evidence indicates that EM2 could serve as the long-sought agonist for the presynaptic μ -opiate receptors on primary afferents. As illustrated in FIGURE 1, the release of EM2 could activate the presynaptic μ receptor to limit the release of excitatory transmitters such as SP. In addition, activation of postsynaptic μ receptors on interneurons and projection neurons within the dorsal horn could decrease the excitability of these neurons. Thus, EM2 may play a major role in the endogenous regulation of the transmission of nociceptive information.

Endomorphin-1 and -2 in Brain

The studies described above established EM2 as a μ agonist localized in circuits involved in the earliest stages of processing nociceptive information. Subsequent mapping studies detailed the immunoreactivity for EM2²⁶ and for both EM1 and EM2²⁷ throughout the nervous system. In general, both EM1- and EM2-LI are present in most areas where either was observed.²⁷ There are striking differences, however, in a few specific areas and in the general pattern of distribution: EM2-LI predominates in the spinal cord and in parts of the medulla. By contrast, EM1-LI is more prevalent in the brain and upper brainstem. There are regional differences between the peptides in the nucleus tractus solitarius (NTS), parabrachial nucleus, and the amygdala. In the NTS, there are large numbers of EM2-LI immunoreactive varicose fibers in the ventrolateral nucleus, whereas EM1-LI is present in cell bodies (visible without colchicine treatment) and in punctate terminal field elements in the dorsomedial nucleus. In the parabrachial nucleus, EM1-LI processes are primarily located in areas lateral to the superior cerebellar peduncle, whereas EM2-LI fibers are predominantly found ventral to it. In the amygdala, EM1-LI processes are present in all nuclei, whereas EM2-LI neuronal elements are relatively confined to the centrolateral nucleus. Thus, although the distributions of the two peptides are very similar, there are some striking differences, possibly indicating either separate precursors or differential processing of a single precursor.

The areas described above that are enriched in endomorphin-LI neuronal elements are also known to contain high densities of μ -opiate receptors and to be in-

volved in the processing of nociceptive information. Other brainstem areas that share these features include the periaqueductal gray, locus coeruleus, nucleus ambiguus, and caudal nucleus of the spinal trigeminal tract. These areas are known to receive primary afferents (e.g., visceral afferents to the NTS) and projection neurons from the dorsal horn, and to serve as relay nuclei to other pain-processing regions. Projection neurons from lamina I of the spinal cord, for example, terminate in the parabrachial nuclei, which in turn convey nociceptive information to the central nucleus of the amygdala. This spino(trigemino)ponto-amygdaloid pathway could modulate emotional/affective, behavioral, and autonomic reactions to noxious stimuli.²⁸ Endomorphin-containing neuronal elements are present in most regions of this pathway.

In addition to areas known to regulate pain, such as the amygdala and midline thalamic nuclei, diencephalic and telencephalic structures enriched in EM-LI indicate a role in neuroendocrine, homeostatic, and limbic functions. After i.c.v. injection of colchicine in the rat, the hypothalamus is the only region where cell bodies are detected except for the NTS (where EM1 cell bodies are found without use of colchicine). Both EM1- and EM2-immunoreactive cells are found in the posterior hypothalamus. Cell bodies for endomorphins may be restricted to these two areas, as β -endorphin cell bodies are restricted to the arcuate nucleus and NTS. It also is possible, however, that the restricted diffusion of colchicine after i.c.v. injection limited the detection of cell bodies to nuclei near the ventricles, and future studies may reveal additional cells that synthesize endomorphins.

Several telencephalic and limbic structures contain both EM-LI fibers and μ -opiate receptors. These include septal nuclei, the diagonal band of Broca, bed nucleus of the stria terminalis, the amygdaloid complex, and many hypothalamic nuclei. In the striatum, the striasomes are rich in μ receptors, but sparse in EM-LI. This area is associated with locomotor effects of opiates and provides a striking example of a mismatch between the μ receptor and endomorphins. At the ventral boundary of this region, however, the nucleus accumbens, which is associated with reward circuitry, contains both μ receptors and many EM1-LI fibers. Some species differences in the distribution of EM-LI exist. For example, EM1-LI fibers in the globus pallidus and cell bodies in the superior olive were detected in mouse but not rat. These results suggest the possibility of some differential functions of EM in the different species.

In summary, the endomorphins are high-affinity endogenous opioids with high selectivity for the μ -opiate receptor. They are potent analgesics and have cellular effects consistent with their μ -agonist profile. Their distribution in many regions of the nervous system containing μ receptors reflects a role as the natural agonists for this receptor. Modulation of pain, autonomic function, and stress responses are functions most likely implicated by the histochemical data, but numerous other functions, including homeostatic, neuroendocrine, and reward processes, also could be modulated by endomorphins.

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REFERENCES

1. HUGHES, J., T.W. SMITH, H.W. KOSTERLITZ, L.A. FOTHERGILL, B.A. MORGAN & H.R. MORRIS. 1975. Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature* **258**: 577–579.
2. LORD, J.A.H., A.A. WATERFIELD, J. HUGHES & H.W. KOSTERLITZ. 1977. Endogenous opioid peptides: multiple agonists and receptors. *Nature* **267**: 495–499.
3. LI, C. H. & D. CHUNG. 1976. Isolation and structure of an untriakontapeptide with opiate activity from camel pituitary glands. *Proc. Natl. Acad. Sci. USA* **73**: 1145–1148.
4. CORBETT, A.D., S.J. PATERSON & H.W. KOSTERLITZ. 1993. Selectivity of ligands for opioid receptors. *In* *Opioids I* (Handb. Exp. Pharmacol. **104/1**). A. Herz, Ed.: 645–679. Springer-Verlag, New York.
5. GOLDSTEIN, A., S. TACHIBANA, L.I. LOWNY, M. HUNKAPILLAR & L. HOOD. 1979. Dynorphin-(1–13), an extraordinarily potent opioid peptide. *Proc. Natl. Acad. Sci. USA* **76**: 6666–6670.
6. CHAVKIN, C., I.F. JAMES & A. GOLDSTEIN. 1982. Dynorphin is a specific endogenous ligand of the κ opioid receptor. *Science* **215**: 413–415.
7. PERT, C.B. 1973. Opiate receptor: its demonstration in nervous tissue. *Science* **179**: 1011–1014.
8. MATTHES, H.W.D., R. MALDONADO, F. SIMONIN, O. VALVERDE, S. SLOWE, I. KITCHEN, K. BEFORT, A. DIERICH, M. LE MEUR, P. DOLLE *et al.* 1996. Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. *Nature* **383**: 819–823.
9. SORA, I., N. TAKAHASHI, M. FUNADA, H. UJIKE, R.S. REVAY, D.M. DONOVAN, L.L. MINER & G.R. UHL. 1997. Opiate receptor knockout mice define μ receptor roles in endogenous nociceptive responses and morphine-induced analgesia. *Proc. Natl. Acad. Sci. USA* **94**: 1544–1549.
10. ZADINA, J.E., A.J. KASTIN, L.-J. GE & L. HACKLER. 1994. Mu, delta and kappa opiate receptor binding of Tyr-MIF-1 and Tyr-W-MIF-1, its active fragments, and two potent analogs. *Life Sci.* **55**: PL461–466.
11. HENSCHEN, A., F. LOTTSPEICH, V. BRANTL & H. TESCHEMACHER. 1979. Novel opioid peptides derived from casein (β -casomorphins). II. Structure of active components from bovine casein peptone. *Hoppe-Seyler's Z. Physiol. Chem.* **360**: 1217–1224.
12. BRANTL, V., C. GRAMSCH, F. LOTTSPEICH, R. MERTZ, K.-H. JAEGER & A. HERZ. 1986. Novel opioid peptides derived from hemoglobin: hemorphins. *Eur. J. Pharmacol.* **125**: 309–310.
13. HORVATH, A. & A.J. KASTIN. 1989. Isolation of tyrosine-melanocyte-stimulating hormone release-inhibiting factor 1 from bovine brain tissue. *J. Biol. Chem.* **264**: 2175–2179.
14. ERCHEGYI, J., A.J. KASTIN & J.E. ZADINA. 1992. Isolation of a novel tetrapeptide with opiate and antioiate activity from human cortex: Tyr-Pro-Trp-Gly-NH₂ (Tyr-W-MIF-1). *Peptides* **13**: 623–631.
15. ZADINA, J.E. & A.J. KASTIN. 1986. Interactions of Tyr-MIF-1 at opiate receptor sites. *Pharmacol. Biochem. Behav.* **25**: 1303–1305.
16. ZADINA, J.E., L. HACKLER, L.-G. GE & A.J. KASTIN. 1997. A potent and selective endogenous agonist for the mu opiate receptor. *Nature* **386**: 499–502.
17. HACKLER, L., J.E. ZADINA, L.-G. GE & A.J. KASTIN. 1997. Isolation of relatively large amounts of endomorphin-1 and endomorphin-2 from human brain cortex. *Peptides* **18**: 1635–1639.
18. HIGASHIDA, H., N. HOSHI, R. KNIJNIK, J.E. ZADINA & A.J. KASTIN. 1998. Endomorphins inhibit high-threshold Ca²⁺ channel currents in rodent NG108-15 cells over-expressing μ -opioid receptors. *J. Physiol.* **507**: 71–75.
19. HARRISON, L.M., A.J. KASTIN & J.E. ZADINA. 1998. Differential effects of endomorphin-1, endomorphin-2, and Tyr-W-MIF-1 on activation of G-proteins in SH-SY5Y human neuroblastoma membranes. *Peptides* **19**: 749–753.

20. MARTIN-SCHILD, S., J.E. ZADINA, A.A. GERALL, S. VIGH & A.J. KASTIN. 1997. Localization of endomorphin-2-like immunoreactivity in the rat medulla and spinal cord. *Peptides* **18**: 1641–1649.
21. ARVIDSSON, U., M. RIEDL, S. CHAKRABARTI, J.-H. LEE, A. NAKANO, R.J. DADO, H.H. LOH, P.-Y. LAW, M.W. WESSENDORF & R. ELDE. 1995. Distribution and targeting of a μ -opioid receptor (MOR1) in brain and spinal cord. *J. Neurosci.* **15**: 3328–3341.
22. MARTIN-SCHILD, S., A.A. GERALL, A.J. KASTIN & J.E. ZADINA. 1998. Endomorphin-2 is an endogenous opioid in primary sensory afferent fibers. *Peptides* **19**: 1783–1789.
23. TUCHSCHERER, M.M. & V.S. SEYBOLD. 1989. A quantitative study of the coexistence of peptides in varicosities within the superficial laminae of the dorsal horn of the rat spinal cord. *J. Neurosci.* **9**: 195–205.
24. PIERCE, T.L., M.D. GRAHEK & M.W. WESSENDORF. 1997. Immunoreactivity for endomorphin-2 occurs in primary afferents in rats and monkey. *NeuroReport* **9**: 385–389.
25. HOLZER, P. 1991. Capsaicin: Cellular targets, mechanisms of action, and selectivity for thin sensory neurons. *Pharmacol. Rev.* **43**: 143–201.
26. SCHREFF, M., S. SCHULZ, D. WIBORNY & V. HOLLT. 1998. Immunofluorescent identification of endomorphin-2-containing nerve fibers and terminals in the rat brain and spinal cord. *NeuroReport* **9**: 1031–1034.
27. MARTIN-SCHILD, S., A.A. GERALL, A.J. KASTIN & J.E. ZADINA. 1999. Differential distribution of endomorphin 1- and endomorphin 2-like immunoreactivities in the CNS of the rodent. *J. Comp. Neurol.* **405**: 450–471.
28. BERNARD, J.F. & J.M. BESSON. 1990. The spino(trigemino)pontoamygdaloid pathway: electrophysiological evidence for an involvement in pain processes. *J. Neurophysiol.* **63**: 473–490.